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CHAPTER 6

Vinca alkaloids

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INTRODUCTION

In this chapter we will review the important developments in the pharmacology of vincristine and vinblastine as well as two new agents, taxol and navelbine, both of which appear to have significant antitumor activity in man.

VINCRISTINE AND VINBLASTINE

The primary site of action of the vinca alkaloids, a reversible binding to tubulin that prevents microtubule formation, has been defined in previous years. The most notable contributions to vinca pharmacology in 1989 concerned the pharmacokinetic and biochemical interaction of these drugs with radiation, cisplatin, and calcium channel blockers. More basic aspects of resistance to the vinca alkaloids are considered as part of the chapter on the multidrug resistance (Chapter 9).

Pharmacokinetics and toxicity

One of the most interesting and immediately relevant papers comes from St. Jude Children's Hospital, where Horton et al. [1] examined the modulation of vincristine pharmacokinetics and toxicity by verapamil in mice. They found that constant infusions of verapamil at a rate of 6.25 mg/kg/h yielded target blood levels of about 10 μM. In vitro studies of the reversal of drug resistance have indicated that the level of verapamil required to reverse resistance varies widely among the various tumors examined, but may be as high as 10 μM. In Horton's work, 10 μM verapamil slowed elimination of vincristine from small intestine, liver and kidney (organs known to express the P-170 glycoprotein), produced neurotoxicity at doses of vincristine that were well tolerated in the absence of verapamil, and lowered the maximal-tolerated dose of vincristine eightfold. Surprisingly, verapamil did not enhance toxicity for a multidrug-resistant human rhabdomyosarcoma cell line, but the mechanism of resistance in this line was not defined. This study warns that reversal agents, particularly when used in high-dose infusions, may augment host toxicity. The few clinical studies to date of vincas combined with verapamil have not encountered such toxicity, but the doses of agents, and particularly verapamil, have been limited by their cardiovascular side effects, and verapamil concentrations in plasma have not exceeded 2 μM. The R isomer of verapamil, and other analogs of calcium channel blockers, might be tolerated at higher doses and could produce significant enhancement of vincristine toxicity.

Cass et al. [2] have reiterated the need for

prolonged exposure to verapamil in attempts at reversal of vincristine resistance. In in vitro experiments with multidrug-resistant cell lines, they showed that 4-h exposure to vincristine and verapamil, with resuspension of cells in drug-free medium, was ineffective in reversing resistance, while resuspension in verapamil inhibited vincristine efflux and enhanced cytotoxicity.

In addition to effects on cellular efflux of vincristine, calcium channel blockers may influence vinca pharmacokinetics. For example, nitrendipine given with vincristine as an intravenous bolus prolonged the plasma half-life of vincristine in 14 patients. More detailed studies of pharmacokinetic interactions in man are clearly needed [3]. These findings are quite different from those of Horton et al. [1], who did *not* find any effect of verapamil on plasma half-life of vincristine in mice, but the drugs and schedules of administration, as well as the species examined, differed in the two reports.

Drug and irradiation interactions

With the growing use of irradiation and drugs in clinical oncology, the variables affecting their interaction need to be defined. Fanelli et al. [4] found that twice-daily irradiation of a murine epithelial carcinoma produced predictable oscillations in tumor uptake of bolus doses of vincristine, with peaks of drug accumulation at days 7, 12 and 20. As the tumor decreased in size, drug accumulation increased. While the study has some obvious shortcomings – including the lack of distinction between parent drug and metabolite, and failure to rule out pharmacokinetic effects – such oscillations are likely due to fluctuations in cell cycle kinetics, and could present opportunities for more rational design of drug-irradiation interaction.

Interactions between cisplatin and vincristine [5] appear to be highly sequence-dependent. When A549 human lung cancer cells are treated first with cisplatin and 6 h later with vincristine, the effect is less than additive, while the opposite sequence gave greater cell kill. Simultaneous exposure to both drugs yielded less than additive results. Current clinical trials give little attention to these apparently antagonistic interactions. Given the frequency with which these drugs are used together, it will be important to examine these interactions more carefully. No explanation is obvious.

The interactions of dipyridamole (DP), a nucleoside transport inhibitor, and the vinca alkaloids were examined in two new reports [6,7]. DP blocked vincristine efflux from *mdr*-type human cancer cell lines through its competitive inhibition of drug binding to the P-170 glycoprotein [6], and potentiated vinblastine cytotoxicity against a drug-sensitive human ovarian carcinoma cell line [7], again by inhibiting drug efflux. It is not clear whether the sensitive line had P-170 membrane protein, although this seems unlikely in view of its sensitivity to vincristine. Howell and colleagues [7] are evaluating dipyridamole interaction with several anticancer drugs in the clinic.

It is worth calling attention to a careful new study of vinblastine's thrombocytopenic effects. By comparison of regimens containing or not containing vinblastine, Steurer et al. [8] convincingly demonstrated that vinblastine produces an acute drop in circulating platelets that reaches a nadir on day 3 and recovers by day 10. Platelet half-life was significantly shortened 24 h after vinblastine; platelets lost their discoid shape within 15 min of drug administration, and ultrastructural studies revealed dissolution of microtubules. These acute effects are independent of the myelosuppressive action of vinblastine.

NAVELBINE

Navelbine (vinorelbine, 3′,4′-didehydro-4′-deoxy-c′-norvincaleukoblastine, Nor-5′-anhy-

drovinblastine) is a semisynthetic vinca alkaloid that inhibits microtubule assembly. It is the only semisynthetic vinca alkaloid in which the catharanthine portion of the molecule rather than the vindoline moiety is modified, yielding an eight-membered catharanthine C′ ring in place of the naturally occurring nine-membered C′ ring. This new structure is more easily protonated with opening of the ring and creation of an ion that will react to produce a covalent reversible bond between navelbine and its target protein tubulin [9].

A series of review articles have been published that summarize current understanding of the mechanism of action, pharmacology, and pharmacokinetics of navelbine (see Seminars in Oncology, Vol. 16, No. 2, Suppl. 4).

Mechanism of action

In the intact tectal plates from mouse embryos, navelbine (NVB), vincristine (VCR) and vinblastine (VBL) are equipotent against mitotic cells – causing depolymerization of microtubules at the same minimal concentration (2.0 μM) – and induced a blockade of cells at metaphase [10]. However, NVB has lesser neurotoxicity than VCR, and this may be explained by differences in their effects on axonal microtubules. VCR produces depolymerization of axonal microtubules at nearly the same concentration (5 μM) that affects mitotic microtubules. In contrast, VBL and NVB require 30 and 40 μM, respectively, to achieve the same effect. Additionally, the neurotoxicity of VCR may result from its effect on MAP and TAU proteins, which are involved with the spiralization of axonal microtubules [11]. NVB only weakly promotes spiralization at equimolar concentrations to VCR and therefore would be expected to have less neurotoxicity at concentrations that produce equal or greater antitumor activity.

Antitumor activity

NVB has demonstrated its efficacy in all tumor models previously known to be sensitive to other vinca alkaloids. It is generally more active than VBL or VCR (except against L1210, where NVB was more active than VCR but less potent than VBL) [12]. The antitumor activity of NVB was not only superior i.v., i.p. and s.c., but *oral* NVB also resulted in significant (greater than 125%) and dose-related increases in survival time in CDF1 mice with P388 engrafted i.p. or i.v. Against a wide variety of human tumor cell lines, NVB was cytostatic at nanomolar concentrations, showing particular activity against non-small-cell lung cancer lines.

Combinations of NVB/VCR, NVB/5-FU and NVB/methotrexate resulted in less than additive antitumor activity but did increase toxicity. Combinations of NVB/doxorubicin, NVB/ifofamide, NVB/cyclophosphamide, NVB/mitomycin-C and NVB/actinomycin-D resulted in neither an increase in antitumor activity nor an increase in toxicity. However, combinations of NVB/etoposide and NVB/cisplatin produced at least additive (and possibly synergistic) effects, with an increase in median survival time and long-term survivors, but no increase in toxicity.

Preclinical toxicology

Toxicity has been studied in rats, dogs and rhesus monkeys on a variety of schedules [13]. The dose-limiting toxicity is hematologic, predominantly neutropenia. No thrombocytopenia has been observed. The dog was the most sensitive species, as with other vinca alkaloids. Reversible elevation of liver enzymes was observed in rats and dogs, but not in monkeys (also reported with other vincas). No neurotoxicity has been observed, even in the monkey, which is particularly sensitive to vinca alkaloid neurotoxicity. The LD_{10} ranged from 1 (dog) to 10 (rodents) mg/kg in

acute toxicity studies. In subacute and chronic studies the maximal-tolerated dose (MTD) was 2 (rat), 0.75 (dog) and 2 (rhesus monkey) mg/kg on the weekly schedule.

Pharmacokinetics and clinical pharmacology

The pharmacokinetics of NVB after i.v. administration are best described by a bi- or tricompartment model, similar to that for other vinca alkaloids [14]. It has a rapid initial clearance rate (0.27 – 1.49 l/h/kg), a large volume of distribution (8.2 – 48.2 l/kg), and a long terminal half-life (22.1 – 67.8 h). Tissue binding appears higher than with other vinca alkaloids, particularly in lung, spleen, stomach and kidney. Elimination studies indicate that NVB is excreted primarily by the fecal route. Unlike other vincas, the oral formulation is reproducibly absorbed, with pharmacokinetic behavior similar to that of the i.v. drug [15]. Absolute bioavailability is approximately 40% in man.

Early clinical trials confirm that leukopenia is dose-limiting for both i.v. and orally administered drug. The MTDs are 30 mg/m^2/week i.v. and 80 mg/m^2/week orally [16]. As in the animal studies, thrombocytopenia has been quite rare. Decrease or loss of deep-tendon reflexes is common after prolonged treatment, but paresthesias, pain or other severe neurotoxicity have been much less common [17 – 19]. Evaluation of both oral and intravenous formulations is under way, with early evidence of activity against breast cancer, non-small-cell lung cancer, and lymphoma.

TAXOL

Taxol is of interest to the oncologist because of its potential as a new antitumor drug, and to the cell biologist because of its unique mechanism of action. Taxol promotes the assembly of stable microtubules that are resistant to depolymerization. Cells treated with taxol develop bundles of stable microtubules that have been observed in a large number of cell types [20].

Antineoplastic activity

Taxol is a promising cancer chemotherapeutic agent that appears to have potential in the treatment of a number of malignancies, particularly ovarian cancer. Both a Phase I study of taxol in refractory acute leukemias and a Phase II study in advanced ovarian epithelial neoplasms have been reported from the Johns Hopkins Oncology Center during 1989. In the Phase I study [21], taxol was administered as a 24-h i.v. infusion to adults with refractory leukemias. The results of this study indicated that the maximum-tolerated doses and recommended Phase II doses for leukemia are 390 and 315 mg/m^2. The major non-hematologic toxicity that limited dose escalation was severe mucositis. Of pharmacologic interest was the development of an assay to assess the sensitivity of a tumor to taxol by taking advantage of the induction of microtubule bundles by taxol. Prior to treatment, leukemic blasts from 12 patients were incubated in vitro with 0.1 to 10 μM taxol for 4 to 24 h and the appearance of microtubule bundles was monitored by immunofluorescence. There was a strong correlation between antitumor activity and the formation of microtubule bundles in leukemia cells incubated with taxol. The leukemia cells of four non-responders did not develop microtubule bundles upon incubation with taxol, while eight patients' blasts demonstrated bundles in vitro and these patients responded to therapy. This assay deserves further evaluation. Mean steady-state plasma taxol concentrations were 1.57 μM at 250 mg/m^2, 2.93 μM at 315 mg/m^2, and 3.50 μM at 390 mg/m^2. No drug was detected in the spinal fluid of a patient whose concurrent plasma level was 2.74 μM at the end of infusion.

The results of the Phase II trial [22] in drug-

refractory ovarian cancer are exciting and potentially very important. The response rate was 30% with myelosuppression being the dose-limiting toxicity. The duration of response in 12 patients was 66 to 462 (median 182) days. The authors point out that a response rate of 30% to taxol, which was being used as a single drug in a pretreated population, was unexpected and remarkable; the results compare favorably to the early studies done with cisplatin in ovarian cancer. A confirmatory Phase II trial is being conducted [23] and the combination of taxol and cisplatin for the treatment of ovarian cancer is being investigated [22]. Since taxol and cisplatin have distinct mechanisms of action, the use of a combination of these two drugs may prove beneficial. The early trials of taxol are discussed in greater detail in the chapter on New Agents in this Annual and Annual 11.

Toxicities

The primary toxicity of taxol is neutropenia, but additional side effects have been reported. A glove-and-stocking sensory neuropathy was described in a series of patients participating in Phase I clinical trials of taxol [24]. Fifty-five percent of patients treated with doses of 200 mg/m^2 or greater developed neuropathic symptoms. The symptoms began in the hands and feet simultaneously after 1 to 3 days of treatment with taxol. Electrophysiologic data suggested that axonal degeneration had occurred. Although the exact mechanism of this neuropathy is not understood, there are many reports indicating that taxol promotes the formation of microtubule bundles in neurons, axons and Schwann cells [25 – 27]. This sensory neuropathy must be anticipated in patients being treated with greater than a 200 mg/m^2 dose of taxol.

Mitotic arrest and cell necrosis in normal host tissues were reported to result from taxol chemotherapy [28]. In biopsy specimens, mitotic arrest was most prominent in the esophagus, but was also observed in the stomach, small intestine, colon, liver and bone marrow. It is not clear why the greatest number of arrested mitoses were seen in the esophageal epithelium, but it could reflect an affinity of taxol for this organ. The arrested mitoses were unusual because the chromosomes were widely dispersed. From the timing of biopsy specimens in relationship to the last treatment with taxol, it was deduced that the mitotic arrest was transient and reversible. Mitotic arrest likely results from the stabilization of microtubules that form and then resolve during normal mitosis.

Synthesis of prodrugs of taxol

There are two problems associated with the development of taxol as an antitumor agent. The compound is a natural product and has been obtained by extraction and purification from the bark of the tree *Taxus brevifolia*. It is also found in other members of this species in low yields, but it has not yet been synthesized. Thus the drug is in short supply for clinical trials. Intensive efforts are under way to develop semisynthetic routes that utilize the drug precursors found abundantly in plants. An alternative is to obtain the final product from the leaves of hedged *Taxus* species rather than from the bark of the tree; however, the parent compound is found in low concentration in leaves.

In addition to problems of supply, taxol is an extremely hydrophobic molecule with low aqueous solubility and lacks functional groups for preparing salts of the drug. Therefore, it has been difficult to develop a good water-soluble formulation. At present it is prepared in polyethoxylated castor oil and absolute ethanol that is diluted with 5% dextrose in water prior to its administration by slow intravenous infusion to patients [29].

Structure-activity studies have indicated that

esterification at C2′ does not result in loss of cytotoxicity [30]. Deutsch et al. [31] have succeeded in preparing a water-soluble prodrug of taxol with potent antitumor activity in experimental tumor systems in mice. Taxol was reacted with glutaric anhydride in pyridine solution to form the mono-2′-adduct, and a salt of this acid was formed. The hydrochloride salt exhibited good aqueous solubility and antitumor activity. This enhancement of solubility is important, but, since the starting material is taxol, it does not alleviate the need to develop new sources of the drug.

Use of taxol as a tool in cell biology

Taxol continues to be extremely useful for the cell biologist because of its singular effects of stabilizing microtubules against depolymerization. For example, in studies with estramustine, taxol played an important role in proving that estramustine inhibits microtubule assembly and disrupts microtubules by binding to MAP-1 and MAP-2, two proteins associated with the microtubular apparatus [32,33]. Estramustine was shown to remove MAP-1 from microtubules stabilized with taxol and to inhibit the binding of MAP-1 to microtubules assembled with taxol.

A detailed study [34] to measure the intrinsic rate of end-to-end joining of microtubules under conditions where subunit exchange was suppressed depended on the use of taxol. Taxol treatment provided stabilized microtubules with only GDP at the 'exchangeable' site. The study indicated that end-to-end joining of microtubules is extremely efficient. An apparent biomolecular rate constant that can be measured under various conditions was described for this joining process.

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